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Review

Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials

F.I. Abdullaev PhD[a,*], J.J. Espinosa-Aguirre PhD[b]

[a]*Laboratorio Oncología Experimental, Instituto Nacional de Pediatría, Avenida Imán # 1 Torre de Investigación, 04530 México D.F., México*

[b]*Instituto de Investigaciones Biomédicas, UNAM, Apartado Postal 70228, Ciudad Universitaria, México D.F., México*

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Abstract

Introduction: Chemoprevention strategies are very attractive and have earned serious consideration as potential means of controlling the incidence of cancer. An important element of anticancer drug development using plants is the accumulation and analysis of pertinent experimental data and purported ethnomedical (folkloric) uses for plants. The aim of this review is to provide an updated overview of experimental in vitro and in vivo investigations focused on the anticancer activity of saffron (*Crocus sativus* L.) and its principal ingredients. Potential use of these natural agents in cancer therapy and chemopreventive trials are also discussed.

Methods: A computerized search of published articles was performed using the MEDLINE database from 1990 to 2004. Search terms utilized including saffron, carotenoids, chemoprevention, and cancer. All articles were obtained as reprints from their original authors. Additional sources were identified through cross-referencing.

Results: Studies in animal models and with cultured human malignant cell lines have demonstrated antitumor and cancer preventive activities of saffron and its main ingredients, possible mechanisms for these activities are discussed. More direct evidence of anticancer effectiveness of saffron as chemopreventive agent may come from trials that use actual reduction of cancer incidence as the primary endpoint

Conclusions: This work suggests that future research be warranted that will define the possible use of saffron as effective anticancer and chemopreventive agent in clinical trials.

Keywords: Saffron (*Crocus sativus*); Cancer chemoprevention; Cytotoxicity; Clinical trials

Contents

* Corresponding author. Tel.: +52 55 10 84 09 00x1474; fax: +52 55 10 84 38 83.
E-mail addresses: fikrat@sni.conacyt.mx, fikrat@servidor.unam.mx, fikrat@yahoo.com (F.I. Abdullaev).

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1. Introduction

Since immemorial times, herbal plants have been used in virtually every culture throughout the world as a source of folk medicine [1,2]. Over two millennia ago, the father of medicine Hippocrates mentioned about 400 medicinal plants and advised, ‘‘let food be your medicine and let medicine be your food’’ [1]. It is still true today and suggests that prevention is more important than treatment. Currently, chemoprevention strategies are very attractive and have earned serious consideration as a potential means of controlling the incidence of cancer [2]. Scientists and medical professionals have shown increased interest in this field, as they recognize the true health benefits of natural remedies. An important element of chemopreventive drug development using plants is the accumulation and analysis of pertinent experimental data and purported ethnomedical (folkloric) uses for plants. It is also very important to note that suitable chemopreventive natural agents should have little or no toxicity, a high efficacy, to be orally administrable, to have a known mechanism of action and of low cost [3]. The present study provides an updated overview of experimental in vitro and in vivo investigations on the biological activities of saffron (*Crocus sativus* L.) and its principal ingredients, especially focusing on their anticancer effect. Potential use of these natural agents in cancer therapy and chemoprevention trials is also discussed.

2. Methods

A computerized search of published articles was performed using the MEDLINE database from the period of 1990–2004. This database, produced by the National Library of Medicine (NLM), includes all medico-biological literature dated from 1974 and so on. Depending on the literature period of interest, it is offered either on-line or off-line. Input data are obtained from approximately 3000 journal titles comprising of life sciences and/or medical information. Approximately, 99% of the data included in the database file are journal articles, of which, 65% are in the English language. The remaining 1% of data represent government documents. Retrieval provides bibliographic data with an abstracted summary, obtained by using keywords and/or subject headings. The search terms utilized including saffron, carotenoids, chemoprevention, and cancer. All articles were obtained as reprints from their original authors. Additional sources were identified through cross-referencing.

3. Results

3.1. Description

Saffron (*Crocus sativus*) is a bulbous perennial of the iris family (Iridaceae) treasured for its golden-colored, pungent stigmas, which are dried and used to flavor and color foods as well as a dye. Saffron is a spice known only in cultivation and principally grown in Spain and Iran, but also cultivated on a lower scale in Greece, Turkey, India, Azerbaijan, France, Italy, India, China, Morocco, Turkey, Israel, Egypt, United Arab Emirates, Mexico, Switzerland, Algeria, Australia, and New Zealand [4,5].

3.1.1. History and folk use

The name saffron comes from the Arabic za’faran, which means yellow. The use of saffron also goes back to ancient Egypt and Rome, where it was used as a dye, in perfumes, and as a drug as well as for culinary purposes [6,7]. As a medicinal plant, saffron has traditionally been considered an anodyne, antidepressant, a respiratory decongestant, antispasmodic, aphrodisiac, diaphoretic, emmenagogue, expectorant, and sedative. It was used in folk remedy against scarlet fever, smallpox, colds, asthma, eye and heart diseases, tumor, and cancer. Saffron can also be used topically to help clear up canquer sores and to reduce the discomfort of teething infants [4,5].

3.1.2. Chemical composition

The stigmas of the saffron flower contain many chemical substances. Carbohydrates, minerals, musilage, Vitamins (especially riboflavin and thiamine) and pigments including crocin, anthocianin, carotene, lycopene, zigzantin, flavonoids, amino acids, proteins, starch, gums, and other chemical compounds have also been described in saffron [4–7].

The saffron stigma, which is what basically forms commercial saffron, has a distinct and unique color, flavor and aroma and some of the groups of chemical compounds responsible for each of these properties have now been identified. One of its principal coloring pigments is crocin, which is easily soluble in water. In addition to crocin, saffron contains crocetin as a free agent and small amounts of the pigment anthocianin, α-carotene, β-carotene, and zegxantin [5,8].

The principal element giving saffron its special ''bitter'' flavor is the glycoside picrocrocin. This bitter tasting substance can be crystallized and produces glucose and the aldehyde safranal by hydrolysis [5,8].

The main aroma factor in saffron is safranal, which comprises of about 60% of the volatile components of saffron. In fresh saffron, this substance exists as a stable picrocrocin but as a result of heat and with the passage of time, it decomposes releasing the volatile aldehyde, safranal [5,8].

3.2. Medical–biological activities of saffron

3.2.1. Toxicity

The toxicity of saffron has been found to be quite low. Animal studies indicate that the oral LD_{50} of saffron was 20.7 g/kg administrated as a decoction [5].

3.2.2. Precautions

Saffron should always be obtained from a reputable source that observes stringent quality control procedures and industry-accepted good manufacturing practices. People with chronic medical conditions should consult with their physician before taking the herb. Pregnant women should never take the herb for medicinal purposes, as saffron can stimulate uterine contractions [9].

3.2.3. Effect on coronary artery disease

Fifty milligrams of saffron dissolved in 100 ml of milk was administered twice a day to human subjects as reported in an Indian study published in 1998. The significant decrease in lipoprotein oxidation susceptibility in patients with coronary artery disease (CAD) indicates the potential of saffron as an antioxidant [10].

3.2.4. Effect on learning behavior and long-term potentiation

Several Japanese studies have reported that the saffron extract and two of its main ingredients crocin and crocetin, improved memory and learning skills in ethanol-induced learning behavior impairments in mice and rats [11–16]. These results suggest that oral administration of saffron may be useful as treatment for neurodegenerative disorders and related memory impairment. Recently, it was shown that crocin isolated from saffron exhibits anti-apoptotic action in PC-12 cells treated with daunorubicin [17]. These findings suggest that crocin inhibits neuronal death induced by both internal and an external apoptotic stimulus in highly differentiated cells (neurons). This selective behavior suggests important therapeutic implications, related to the fact that programmed cell death is reduced in cancer and increased in neurodegenerative disease [17].

3.2.5. Effects on ocular blood flow and retinal function

It was shown that crocin analogs isolated from saffron significantly increased the blood flow in the retina and choroid as well as facilitated retinal function recovery [18]. Authors suggest that crocin analogs could be used to treat ischemic retinopathy and/or age-related macular degeneration.

3.2.6. Effect on blood pressure

Recently, an Iranian study researches examined the effects of saffron petal extract on blood pressure in anesthetized rats and on responses of the isolated rat vas deferens and guinea-pig ileum induced by electrical field stimulation (EFS). It was shown that aqueous and ethanol extracts of saffron reduced the blood pressure in a dose-dependent manner. EFS of the isolated rat vas deferens also were decreased by these saffron extracts [19].

3.2.7. Anticonvulsant effect

In Iranian traditional medicine, the saffron had been used as an anticonvulsant remedy. Recently, in experiments with mice using maximal electroshock seizure (MES) and pentylenetetrazole (PTZ) tests, Iranian scientists have demonstrated that the aqueous and ethanolic extracts of saffron possess anticonvulsant activity. These authors suggested that saffron extracts might be beneficial in both absence and tonic clonic seizures [20].

3.2.8. Antinociceptive and anti-inflammatory effects

An Iranian experimental study with mice indicated that saffron stigma and petal extracts exhibited antinociceptive effects in chemical pain test as well as acute and/or chronic anti-inflammatory activity [21]. It was suggested that these effects of saffron extracts might be due to their content of flavonoids, tannins, anthocyanins, alkaloids, and saponins [22].

3.2.9. Mutagenic or antimutagenic effects

It was reported that crocin and dimethyl-crocetin isolated from saffron were non-mutagenic [23]. Recently, data from our laboratory, using the Ames/*Salmonella* test system (strains TA97; TA98; TA100; TA102, and TA1538),

demonstrated that the saffron extract itself in concentration up to 1500 μg/plate was non-toxic, non-mutagenic, and non-antimutagenic [24,25].

3.2.10. Antigenotoxic effect

It was reported that the topical administration of saffron extracts (100 mg/kg body weight) inhibited the initiation/promotion of 7,12-dimethylbenz [a] anthracene (DMBA)-induced skin tumors in mice, delaying the onset of papilloma formation and reducing the mean number of papillomas per mouse [26]. The oral administration of the same dose of saffron extracts restricted tumor incidence of 20-methyl-cholanthrene (MCA)-induced soft tissue sarcomas in mice [23,26]. Extracts from saffron stigmas prolonged the life span of cisplatin-treated mice and partially prevented the decrease in body weight, leukocyte count and hemoglobin levels [27–29].

Pretreatment with the aqueous extract of saffron (composed mainly by carotenoids) in experiments with Swiss albino mice significantly inhibited the genotoxicity of cisplatin, cyclophosphamide, mitomycin, and urethane [30]. It was suggested that saffron rich in carotenoids might exert its chemopreventive effects by the modulation of lipid peroxidation, antioxidants, and detoxification systems [31]. Crocetin from saffron also ameliorates bladder toxicity of the anticancer agent cyclophosphamide without altering its antitumor activity [28].

The treatment of animals with cysteine (20 mg/kg body weight) together with saffron extract (50 mg/kg body weight) significantly reduced the toxic effects caused by cisplatin, such as nephrotoxicity and changes in enzyme activity [32].

3.2.11. Tumoricidal effect

It has been previously shown that saffron was more active parenterally than by oral route, and oral administration might be improved by the liposome encapsulation of the drug. It was reported that the liposome encapsulation of saffron produced a significant inhibitory effect on the growth of transplanted tumor cells in mice [33]. Recently, in an animal model (frog embryos), it was demonstrated that crocetin, isolated from saffron was effective in treating certain types of cancer treatable with all-trans retinoic acids (ATRA). It was suggested that crocetin might also be a safer alternative to treat ATRA-sensitive cancers in women of childbearing age [34].

The oral administration of the saffron ethanolic extract increased the life span of Swiss albino mice intraperitoneally transplanted with sarcoma-180 (S-180) cells, Ehrlich ascites carcinoma (EAC) or Dalton's lymphoma ascites (DLA) tumors. The authors did not identify the exact nature of the active compound from saffron stigmas, but suggested that this compound showed the presence of glycosidic linkage. Liposome encapsulation of saffron effectively enhanced its antitumor activity against S-180 and EAC solid tumors in mice, promoting significant inhibition in the growth of these tumors [35,36]. On the other hand, in the presence of the T cell mitogen phytohemagglutinin, saffron stimulated non-specific proliferation of lymphocytes in vitro [36], suggesting that the antitumor activity might be immunologically mediated. Another study [37] examined the effects of long-term treatment with crocin on tumor growth and life span of rats bearing colorectal tumors, induced by rat adenocarcinoma DHD/K12-PROb cells injected subcutaneously. Crocin treatment significantly increased their survival time and decreased tumor growth rate, more intensely in females. The selective action of crocin in female rats as compared with male rats suggests that the effects of crocin in animals might be partially dependent on hormonal factors. An increase in the levels of β-carotene and Vitamin A in the serum of laboratory animals under oral administration of saffron extracts was detected [32]. It was suggested that saffron carotenoids possessed provitamin A activity according to the hypothesis that the action of carotenoids was dependent upon its conversion to retinal (Vitamin A), because most of the evidence supporting the anticancer effects of carotenoids were referred to β-carotene [38].

3.2.12. Cytotoxic effect

Incubation of HeLa cells (derived from a cervical epitheloid carcinoma) with ethanolic saffron extract resulted in significant inhibition of colony formation and cellular DNA and RNA synthesis, with 50% inhibition obtained at concentrations from 100 to 150 μg/ml, whereas inhibition of protein synthesis was not detected even at high extract concentrations [39]. In other study on the effect of the ethanolic saffron extract on macromolecular synthesis in three human cell lines: A549 cells (derived from a lung tumor), WI-38 cells (normal lung fibroblasts) and VA-13 cells (WI-38 cells transformed by SV40 virus), it was found that the malignant cells were more sensitive than the normal cells to the inhibitory effects of saffron on both DNA and RNA synthesis [40]. It has been suggested that the inhibitory effect on cellular DNA and RNA synthesis, but not on protein synthesis, is one of the main mechanisms of action for saffron's antitumor and anticarcinogenic activities [5,36,39–41]. The inhibitory effect of crocetin, isolated from saffron, on intracellular nucleic acid and protein synthesis in three malignant human cell lines, HeLa, A549 (lung adenocarcinoma), and VA13 (SV-40 transformed foetal lung fibroblasts) was reported [41]. Crocetin caused a dose-dependent inhibition of nucleic acid and protein synthesis, but had no effect on colony formation. Other studies described the inhibition of growth of human chronic myelogenous leukaemia K562 and promyelocytic leukaemia HL-60 cells by dimethyl-crocetin, crocetin, and crocin with 50% inhibition (ID_{50}) reached at concentrations of 0.8 and 2 μM, respectively, [38,42]. Cytotoxicity of dimethyl-crocetin and crocin to various tumors cell lines (DLA, EAC, S-180, L1210 leukemia, and P388 leukemia) and to human primary cells from surgical specimens (osteosarcoma, fibrosarcoma, and ovarian carcinoma) has been reported.

These authors also detected significant inhibition in the synthesis of nucleic acids, and suggested that dimethylcrocetin could disrupt DNA-protein interactions (e.g. toposiomerases II) important for cellular DNA synthesis [26,36].

The inhibitory effect of the ethanolic saffron extract on the in vitro growth of HeLa cells (ID_{50} = 2.3 mg/ml) was mainly due to crocin (ID_{50} of 3 μM), where picrocrocin and safranal, with an ID_{50} of 3 and 0.8 mM, respectively, played a minor role in the cytotoxicity of saffron extracts [43]. It was suggested that sugars might play a key role in cytotoxic effect of crocin, since its deglucosylated derivative crocetin did not cause cell growth inhibition even at high doses. These findings are in accordance with the results [42], which found no effect of crocetin on colony formation in HeLa cells and two other solid tumor cell lines. However, they are in disagreement with results from other authors who reported cytotoxicity for crocetin against a cell line derived from a non-solid tumor [38] and various tumor cell lines and human primary cells from surgical specimen [34]. An ID_{50} of 0.4 and 1.0 mM was reported for crocin on the rat adenocarcinoma DHD/K12-PROb cells and human colon adenocarcinoma HT-29 cells, respectively [37].

In other study using saffron, ginsenoside, and cannabinoid derivatives to determine potential membrane-associated antitumor effects of these substances, it was demonstrated that saffron derivatives were ineffective on the reversal of multidrug resistance of lymphoma cells (the reversal of multidrug resistance is the result of the inhibition of the efflux pump function in the tumor cells) [44]. Microscopy studies revealed that HeLa cells treated with crocin exhibited vacuolated areas, size reduction, cell shrinkage, and piknotic nuclei [37,43], suggesting that programmed cell death is induced by crocin, as was previouly proposed by Morjani [42]. A remarkable bioactive agent has been isolated from the corms of the saffron plant [45,46]. This agent showed an ID_{50} of 9 μg/ml against HeLa cells. The cytotoxic activity of this agent on human malignant cell lines (HeLa, breast carcinoma MDA-MB-231, and fibrosarcoma HT-1080), a non-malignant cell line (fibroblasts ASJ-4), and blood cells and hair follicles in culture, was also analyzed. ID_{50} values ranged from 7 to 22 μg/ml for tumor cells, and 100 μg/ml for normal fibroblasts. Comparison of ID_{50} values for fibrosarcoma cells and normal fibroblasts, both of mesenchymal origin, showed that this agent is near eight times more toxic on tumor cells than on non-tumor cells [47].

4. Conclusions

Saffron *Crocus sativus* L. and their associated carotenoid ingredients are extensively studied for their biomedical properties, especially for their chemopreventive potential against cancer, during the last decade [5–7,25,36,48–50]. Since ancient times, saffron was used in folk medicine as an anticancer agent against different kinds of tumors and cancers [49,50]. In the early 1990s, Indian studies and our own have demonstrated that crude saffron extracts present antitumor and anticarcinogenic activities as well as cytotoxic and antimutagenic effects [35,39,40]. A number of in vivo and in vitro experiments discussed above and in our recent review [5] clearly indicate that saffron and its main ingredients have the potential to reduce the risk of developing several types of cancer. Different hypotheses for the mode of anticancer action of saffron and its ingredients have been proposed (Table 1) and in detail discussed in our previous review [5]. Recently, it was reported that three new monoterpenoids and a new naturally occurring acid were isolated from methanol extracts of the petals of saffron. Among them, crocusatin H, crocin-1, and crocin-3 showed significant tirosine inhibitory activity [51,52]. Iranian scientists have demonstrated that three L-lactate dehydrogenase termostable isoenzymes were detected in saffron corms [53–54]. To date, the exact mechanism of anticancer effect of saffron is not clear. However, all of the available information from animal and in vitro studies indicated that saffron and their main constituents possess anticancer and antitumor activities. These findings have not yet been verified by clinical trials in humans. Comprehensive, in-depth studies still need to be conducted to define the mechanisms involving in the therapeutic properties of saffron and in addition to performing clinical trials in humans. Indoor cultivation and modern biotechnological

Table 1
Proposed mechanism of chemopreventive agent actions

Chemopreventive agents	Mechanism of action	Reference
Saffron extract	Inhibition of itracellular nucleic acid synthesis	[4,5,34]
Saffron extract and its carotenoids ingredients	Inhibition of free radical chain reaction	[5,30,33,35]
Saffron extract	Stability to irradiation	[49]
Carotenoids	Metabolic conversion of carotenoids to retinoids	[5,38]
Carotenoids	Reaction with topoisomerase II	[33,35]
Carotenoids	Blocked the cytochrome C activation of caspase-3	[21]
Saffron extract and its carotenoids ingredients	Increase of intracellular SH compounds	[33,38,39]
Saffron extract	Inhibition of genotoxicity	[29,31–33]
Saffron extract and its carotenoids ingredients	Induction of apoptosis	[5,41,42]
Saffron extract and its carotenaoids ingredients	Inhibition of different cellular enzymes activity	[30,34,43]
Saffron extract and its carotenoids ingredients	Inhibition of cell proliferation	[5,35,37,42]

methods will prove advantageous in achieving the largest amount and highest quality of saffron as well as in reducing its cost of production. In the continued search for new antitumor agents, investigators dedicate their efforts to the study of natural compounds and their effects in modifying cancer risks, delaying carcinogenesis, or inhibiting tumor formation. This work suggests that future research be guaranteed to evaluate the possible use of saffron as an effective anticancer agent in clinical trials.

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